Send Orders for Reprints to reprints@benthamscience.ae

218 *Current Alzheimer Research,* **2015,** *12,* **218-227**

Identification of Novel Quantitative Traits-Associated Susceptibility Loci for *APOE* ε4 Non-Carriers of Alzheimer's Disease

Shan Jiang[1], Wanling Yang[2], Yu Qiu[1,*], Hong-Zhuan Chen[1,*] and for the Alzheimer's Disease Neuroimaging Initiative (ADNI)[#]

Yu Qiu

[1]*Department of Pharmacology, Institute of Medical Sciences, Shanghai Jiao Tong University School of Medicine, 280 South Chongqing Road, Shanghai 200025, China;* [2]*Department of Paedia trics and Adolescent Medicine, LKS Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong*

Hong-Zhuan Chen

Abstract: *APOE* ε4 allele is a major risk factor in Late-Onset Alzheimer's Disease (AD). Distinct phenotypes that depend on the *APOE* ε4 status have been demonstrated. The genetic etiology of *APOE* ε4 non-carriers is still elusive. Thus we investigated the genetic components of AD that is independent of *APOE* ε4 by combining genome association analysis with quantitative trait analyses in non-Hispanic Caucasian participants in the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort. Five top susceptible single nucleotide polymorphisms (SNPs) in three loci in *ZNF827*, *KDM2B* and *NANP* were initially identified in *APOE* ε4 non-carriers and four of these SNPs were confirmed in mild cognitive impairment. These SNPs and one nominally significant SNP are located in three haplotype blocks. Quantitative trait analyses of these haplotype blocks demonstrated that the haplotype block in *ZNF827* was associated with CSF $A\beta_{42}$ level, and the haplotype block in *KDM2B* with CSF p-tau_{181p} and p-tau_{181p}/$A\beta_{42}$ ratio. The haplotype block between *NANP* and *NINL* was associated with brain atrophy. Moreover, these SNPs took additive effects on AD incidence and demonstrated the interaction with *APOE* ε4 status. Therefore, we conclude that these novel loci are associated with AD in *APOE* ε4 non-carriers. This study indicates the distinct genetic risk genes for AD non-carrying *APOE* ε4 and provides new insight into the molecular mechanisms of AD.

Keywords: Alzheimer's disease, apolipoprotein E ε4, brain atrophy, diagnostic biomarker, haplotype, single nucleotide polymorphism.

INTRODUCTION

Alzheimer's Disease (AD) is the most common form of dementia with strong genetic etiology. Apolipoprotein E (*APOE*) ε4 allele has been universally confirmed as a risk factor in Late-Onset Alzheimer's Disease (LOAD) [1, 2]. However, *APOE* ε4 only accounts for 50-65% of LOAD cases, with genetic risk factors of as much as 50% of patients remained unresolved [1, 3].

APOE ε4 differentially affects disease progression as compared to ε2 and ε3 alleles [4]. Patients of *APOE* ε4 carriers perform worse in memory tasks [5], while patients of *APOE* ε4 non-carriers perform worse in naming, mental speed and executive function [6, 7]. A positron emission tomography study demonstrates that *APOE* ε4 carriers and non-carriers show reduced perfusion in different brain areas during a working memory task [8]. Moreover, several genes have been suggested to be differentially involved in the development and pathology of AD in *APOE* ε4 carriers and non-carriers [9-12]. In *APOE* ε4 carriers, the V allele of residue 405 of cholesteryl ester transfer protein (CETP) which decreases CETP and increases HDL is associated with less brain atrophy. However, the I allele, which increases CETP and decreases HDL, is associated with less brain atrophy and lower risk of dementia in *APOE* ε4 non-carriers [9]. Some genes such as cystatin C and *TNFRSF6* are associated with AD only in *APOE* ε4 carriers [11, 12]. Recently, validation of identified predisposition genes has shown that some genes, such as *PICALM* and *CUGBP2*, are affected by correlation with *APOE* [13]. Furthermore, Rhinn *et al.* demonstrated that regulatory molecules and pathways could be involved in the pathology of AD dependent on *APOE* ε4 status [14]. Regulatory molecules, such as *RNF219* and *SV2A*, can modulate APP proteolytic processing and localization in an *APOE* ε4-dependent manner [14]. In addition, several single nucleotide polymorphisms (SNPs) within *APOE* promoter are reported to affect promoter activity and correlate with AD [15, 16]. These SNPs can be modulated by environmental factors, such as heavy metals and high cholesterol diet, thus leading to the development of the disease epigenetically [17].Therefore, to uncover new predisposition genes based on stratification of *APOE* ε4 status may be im-

*Address correspondence to these authors at the Department of Pharmacology, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China; Tel: +86-21-63846590-776451; Fax: +86-21-64674721; E-mails: yu_qiu@hotmail.com and hongzhuan_chen@hotmail.com

[#]*Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.ucla.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.ucla.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf*

1875-5828/15 $58.00+.00

portant. One study demonstrated that a SNP from *GRB*-associated binding protein 2 modifies the risk of AD in *APOE* ε4 carriers [18]. Another study also revealed some differential susceptibility loci in *APOE* ε4 and non-carriers [19]. However, the study aiming to identify the susceptibility loci in *APOE* ε4 non-carriers is scarce.

β-amyloid peptides (Aβ, especially $A\beta_{42}$), total tau (t-tau) and phosphorylated tau (especially phosphorylation of Thr181, p-tau_{181p}) in cerebrospinal fluid (CSF) are diagnostic biomarkers for AD [20]. $A\beta_{42}$ is reduced, and t-tau and p-tau are enhanced in CSF of AD patients. Other quantitative traits such as brain morphometric volume are also reported. Some SNPs associated with AD are found to influence these quantitative traits, which may implicate the pathological role of them [21, 22], however, most of them are not identified through genome-wide association study (GWAS). On the other hand, many AD-associated SNPs identified through GWAS [23, 24] have not been shown to correlate with diagnostic biomarkers and other quantitative traits of AD.

The Alzheimer's Disease Neuroimaging Initiative (ADNI), a multi-centered project with combined genotyping, CSF markers and serial magnetic resonance imaging (MRI) scans, provides valuable data to assess whether the SNPs not only show genome association but also associate with diagnostic or characteristic indices of AD. In present study, we combined genome association analysis with quantitative trait analyses to identify susceptible SNPs in AD, especially in *APOE* ε4 non-carriers.

MATERIALS AND METHODS

Alzheimer's Disease Neuroimaging Initiative (ADNI)

Data used in the preparation of this article were obtained from ADNI database (www.loni.ucla.edu\ADNI). The ADNI was launched in 2003 by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, the Food and Drug Administration, private pharmaceutical companies and non-profit organizations, as a $60 million, 5-year public-private partnership. The primary goal of ADNI has been to test whether serial MRI, PET, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of Mild Cognitive Impairment (MCI) and early AD. Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials.

The Principal Investigator of this initiative is Michael W. Weiner, M.D., VA Medical Center and University of California – San Francisco. ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the U.S. and Canada. The initial goal of ADNI was to recruit 800 adults, ages 55 to 90, to participate in the research -- approximately 200 cognitively normal older individuals to be followed for 3 years, 400 people with MCI to be followed for 3 years, and 200 people with early AD to be followed for 2 years. For up-to-date information see www.adni-info.org.

ADNI was performed in accordance with the Declaration of Helsinki and approved by the Institutional Review Boards of all of the participating institutions. Informed written consent was obtained from all participants at each site [25, 26].

Participants

We included 394 AD and cognitively healthy older controls of non-Hispanic Caucasians aged 55 to 90, recruited from over 50 observational sites across the United States and Canada. Of these 394 participants, 180 were AD patients and 214 healthy control subjects, and about 96 AD patients and 109 healthy controls own fully examined clinical, CSF biomarker, neuropsychological, MRI and *APOE* genotyping data. We also included 165 MCI patients who were *APOE* ε4 non-carriers in ADNI database.

Sampling of CSF and *APOE* Genotyping

APOE genotyping was performed at the time of participant enrollment (screening level). CSF biomarkers data at screening and 12-month and 36-month visits were collected through lumbar puncture. The determinations of *APOE* and biomarkers were carried out by Drs Leslie Shaw and John Trojanowski of the ADNI Biomarker Core at the University of Pennsylvania School of Medicine (for more information please visit http://www.adni-info.org).

SNP Genotyping

The genome-wide SNP dataset was downloaded from ADNI website. All genomic DNA samples were analyzed on the Human610-Quad BeadChip (Illumina, Inc. San Diego, CA) according to the manufacturer's protocols (Infinium HD Assay; Super Protocol Guide; Rev. A, May 2008).

MRI Acquisition

All sites which met all the requirements for the Alzheimer Disease Cooperative Study start-up have completed the MRI certification for 1.5T MRI. All the scans were reviewed for quality control by staff in the ADNI MRI quality control center at Mayo Clinic. Detailed MRI acquisition and processing are described in previous publications [27, 28]. Furthermore, system specific corrections of certain image artifacts were performed [28].

Statistical Analysis

Allelic association test was applied to assess whether these qualified SNPs were associated with AD using the χ^2 statistic. False Discovery Rate (FDR) correction was applied for multiple comparisons. Linkage disequilibrium (LD) indices, r^2 and D', were utilized to assess the linkage status between these identified SNPs. And if r^2 value is more than 0.9 within two or more SNPs, they were considered to be completely linked. And the SNP at the forefront physical location of the haplotype block was chosen to represent the other SNPs in the haplotype block. Manhattan plots were generated in Haploview (http://www.broadinstitute.org/haploview/haploview), release v 4.2 [29]. Multidimensional scaling (MDS) analysis was employed to assess population structures of case and control groups and outliers in software package PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/), release v 1.07 [30], and R language, release v 2.15.2, was used to generate MDS plots. Skewing CSF and brain morphomet-

ric biomarker data were log-transformed before applying student-*t* test or analysis of variance (ANOVA). Levene statistic was used to evaluate the homogeneity of variances before ANOVA. The normality of distribution was assessed using the Kolmogorov-Smirnov test. The most fitted genetic model was chosen for each quantitative trait variable. That is to say, in cases where the additive model was significant, the dominant and recessive models were tested to determine whether they were a better fit. Multivariate logistic regression analysis was utilized to assess the predicting powers of identified statistically significant quantitative trait-associated haplotypes, and pseudo-R^2 was the index for evaluating goodness of fit. To evaluate the interaction between identified SNPs and *APOE* ε4, multivariate logistic regression analysis was utilized with the following model, *Phenotype = a + b.SNP + c.APOE ε4 status + d.Sex + e.Age + f.Education years + g. SNP×APOE ε4 status*. Kaplan-Meier plots were used to describe the relationships between onset of AD symptoms and different genotypes or haplotypes among different SNPs or haplotype blocks. $P < 0.05$ was considered statistically significant.

RESULTS

Screening of Candidate SNPs for Quantitative Trait Analyses Based on *APOE* ε4 Status

Demographic characteristics of normal controls and AD patients were compared (Table **1**), no significant differences were found in variables like baseline age, sex and education. Further, MDS plots showed that cases and controls were matched well and there were no outliers (Fig. **1A**).

Standard SNP quality control was carried out using PLINK. Given the relatively small sample size and in order to reduce the false positive rate, we only included SNPs with minor allele frequency (MAF) greater than 20%. Maximum SNP missing rate was set at 10%, and Hardy-Weinberg Equilibrium was required at $P > 0.001$. SNPs were then tested for allelic association applying χ^2 test without considering the *APOE* ε4 status. False discovery rate (FDR) of 5% is usually utilized to obtain meaningful SNPs. Nevertheless, overstrict threshold may leave out many genes that play important roles and are associated with quantitative traits, and many genes under an overstrict threshold can gather together to play central roles for complicated chronic diseases [31]. FDR of 20% has been universally utilized in many genome association studies encountering multiple test in order to include more SNPs [32-34]. And we were aiming to screen candidate SNPs for quantitative trait analyses, therefore, a relatively liberal FDR of 20% was set in the present study to obtain a moderate amount of nominally significant SNPs. Moreover, multiple dataset correction was attained by multiplying the original *P*-value by three (the total count of all datasets analyzed: all participants group, *APOE* ε4 carriers sub-group and *APOE* ε4 non-carriers sub-group as stated below). Among qualified 326,482 SNPs, two candidate SNPs, rs2075650 (P value = 5.8×10^{-8} and OR = 2.56 with 95% CI of 1.82-3.6) and rs12822144 (P value = 1.14×10^{-6} and OR = 0.49 with 95% CI of 0.37-0.65) were identified (Fig. **2A**, Table **2**). rs2075650, located within the *TOMM40* gene, is a previously reported SNP associated with AD and is in linkage disequilibrium (LD) with the *APOE* ε4 variant on chromosome 19 [23]. rs12822144 is a SNP located within the intergenic region between *RPL18P9* and *CCND2*.

AD patients carrying one *APOE* ε4 allele demonstrate similar clinical and pathological manifestations to that of two ε4 alleles AD carriers and it is the same situation in *APOE* ε4 non-carriers [35-37], thus we stratified the cohort according to *APOE* genotypes: *APOE* ε4 carriers which carried one or two *APOE* ε4 alleles and *APOE* ε4 non-carriers. No significant differences were found in demographic characteristics of normal controls and AD patients in these subgroups (Table **1**). Further, MDS plots showed that cases and controls were matched well and there were no outliers in *APOE* ε4 carriers (data not shown) and non-carriers (Fig. **1B**). Because of different MAFs of many SNPs between *APOE* ε4 carriers and non-carriers, quality control was performed again for each subgroup. No candidate SNP was identified in *APOE* ε4 carrier subgroup after FDR correction for the qualified 325,123 SNPs. For the qualified 327,099 SNPs in *APOE* ε4 non-carriers, five candidate SNPs, rs6816078 (P value = 4.34×10^{-8} and OR = 0.27 with 95% CI of 0.17-0.44), rs28604990 (P value = 1.01×10^{-7} and OR = 3.22 with 95% CI of 2.08-5.01), rs11930385 (P value = 2.89×10^{-7} and OR = 0.30 with 95% CI of 0.18-0.48), rs7955747 (P value = 2.38×10^{-6} and OR = 2.99 with 95% CI of 1.88-4.76) and rs2387976 (P value = 2.92×10^{-6} and OR = 0.32 with 95% CI of 0.19-0.52) were identified (Fig. **2B**, Table **2**). The last SNP rs6076364 showed in Table **2** was slightly below the threshold of FDR = 20% but was in complete LD with the

Table 1. Demographic characteristics of participants for GWA analysis.

	All Participants				*APOE* ε4 Carriers				*APOE* ε4 Non-Carriers			
Variables	**Controls (n=214)**	**AD Patients (n=180)**	**Stastistical Test**	***P* Value**	**Controls (n=58)**	**AD Patients (n=120)**	**Stastistical Test**	***P* Value**	**Controls (n=156)**	**AD Patients (n=60)**	**Stastistical Test**	***P* Value**
Baseline age, y, mean ±SEM	74.48 ± 0.49	74.16 ± 0.53	t = 0.427	0.669	76.07 ± 0.91	75.25 ± 0.60	t = 0.762	0.447	73.88 ± 0.58	71.89 ± 1.01	t = 1.749	0.082
Female, %	46.7	43.2	χ^2 = 0.470	0.493	47.1	39.5	χ^2 = 1.461	0.227	45.8	50.9	χ^2 = 0.438	0.508
Education, y, mean±SEM	15.84 ± 0.19	15.97 ± 0.21	t = -0.452	0.652	16.00 ± 0.34	16.10 ± 0.26	t = -0.230	0.818	15.78 ± 0.23	15.70 ± 0.39	t = 0.187	0.852

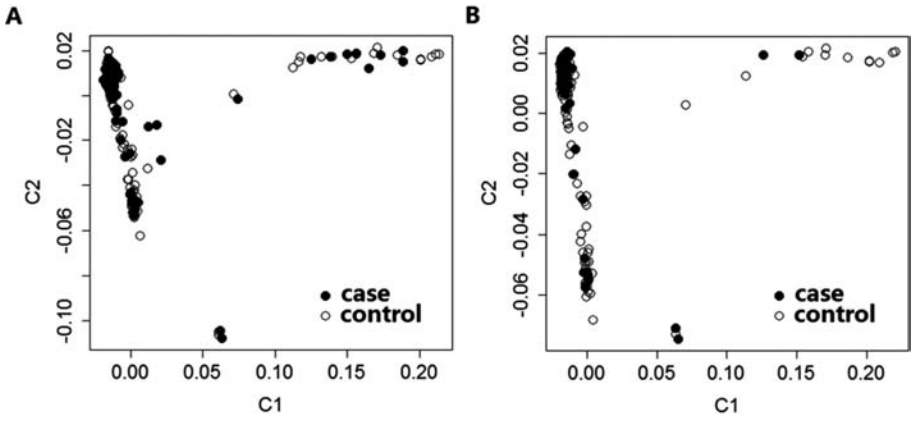

Fig. (1). MDS plots for all participants (**A**) and *APOE* ε4 non-carriers subgroup show no evident population stratification and outliers.

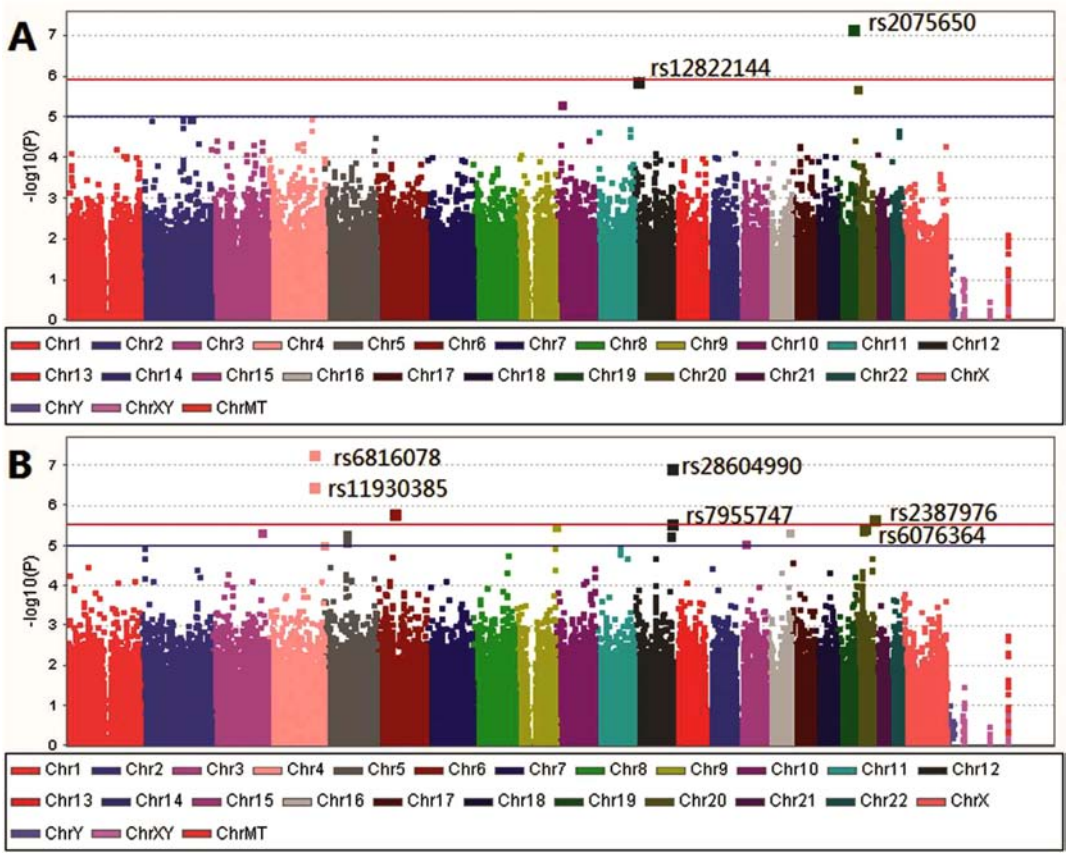

Fig. (2). Manhattan plots of candidate SNPs for all participants (**A**) and *APOE* ε4 non-carriers (**B**). **A**: The threshold of FDR = 20% for genome-wide significance ($P \leq 1.225\times10^{-6}$) is indicated by red horizontal line, and the less stringent threshold for probable association with AD ($P \leq 1\times10^{-5}$) is indicated by blue horizontal line. **B**: The threshold of FDR = 20% for genome-wide significance ($P \leq 3.057\times10^{-6}$) is indicated by red horizontal line, and the less stringent threshold for probable association with AD ($P \leq 1\times10^{-5}$) is indicated by blue horizontal line. The plots were generated by using Haploview 4.2.

significant SNP rs2387976. rs6816078 and rs11930385 are located within a haplotype block in intron 1 of *zinc finger protein 827* (*ZNF827*), rs28604990 and rs7955747 are located within a haplotype block that encompasses intron 6 and intron 12 of *lysine-specific demethylase 2B* (*KDM2B*), and rs2387976 and rs6076364 are located within a haplotype block that contains parts of *N-acetylneuraminic acid phosphatase* (*NANP*) and *ninein like protein (NINL)*. LD indices were r^2 = 0.908 and D' = 0.978 for rs6816078 and rs11930385, r^2 = 0.421 and D' = 0.908 for rs28604990 and rs7955747, and r^2 = 1 and D' = 1 for rs2387976 and rs6076364. Because some genotypic data on rs2387976 and rs6076364 were not detected in some individuals and the individuals missing these two SNPs were different, *P* values of allelic association tests on these two SNPs were slightly different even if they were completely linked (rs2387976 was above the threshold of FDR = 20% and rs6076364 was slightly below the threshold, Fig. (**2B**) and Table **2**).

Table 2. Identified SNPs in AD and MCI patients in all participants and *APOE* ε4 non-carriers[a].

Group	SNP	Chr.	Closest RefSeq gene	Location Relative to Gene	Risk allele	Frequency in Controls	Frequency in MCI Patients	Frequency in AD Patients	*P* value for MCI Patients	OR (95% CI) for MCI Patients[b]	*P* value for AD Patients	OR (95% CI) for AD Patients[b]	Adjusted *P* Value for AD Patients[c]	Adjusted OR (95% CI) for AD Patients[b c]
All participants	rs2075650	19	TOMM40	Intron 1	G	0.150	0.265	0.311	5.65×10^{-6}	2.05 (1.50-2.80)	5.8×10^{-8}	2.57 (1.82-3.63)	1.57×10^{-7}	2.66 (1.84-3.83)
	rs12822144	12	CCND2	Intergenic region	G	0.440	0.541	0.614	8.71×10^{-4}	0.66 (0.52-0.85)	1.14×10^{-6}	0.49 (0.37-0.65)	4.29×10^{-5}	0.54 (0.40-0.72)
APOE ε4 non-carriers	rs6816078	4	ZNF827	Intron 1	C	0.474	0.597	0.767	0.002	0.61 (0.45-0.83)	4.34×10^{-8}	0.27 (0.17-0.44)	3.55×10^{-6}	0.31 (0.19-0.51)
	rs28604990	12	KDM2B	Intron 6	A	0.327	0.441	0.610	0.003	1.63 (1.18-1.25)	1.01×10^{-7}	3.22 (2.08-5.01)	2.65×10^{-5}	2.79 (1.73-4.49)
	rs11930385	4	ZNF827	Intron 1	C	0.494	0.600	0.767	0.007	0.65 (0.48-0.89)	2.89×10^{-7}	0.30 (0.18-0.48)	3.45×10^{-6}	0.29 (0.17-0.49)
	rs7955747	12	KDM2B	Intron 12	A	0.192	0.277	0.415	0.013	1.61 (1.10-2.35)	2.38×10^{-6}	2.99 (1.88-4.76)	1.50×10^{-4}	2.60 (1.58-4.25)
	rs2387976	20	NANP	Intron 1	C	0.524	0.547	0.776	0.575	0.91 (0.66-1.25)	2.92×10^{-6}	0.32 (0.19-0.52)	3.91×10^{-5}	0.33 (0.19-0.56)
	rs6076364	20	NINL	Intron1	T	0.523	0.546	0.767	0.559	0.91 (0.67-1.24)	3.78×10^{-6}	0.33 (0.21-0.54)	2.86×10^{-5}	0.33 (0.19-0.55)

[a]Abbreviation: Chr, chromosome; OR, odds ratio; CI, confidence interval.
[b]OR values were computed for minor alleles.
[c]*P* values and OR values were adjusted for age of onset, sex and education.

The analysis of rs28604990-rs7955747 showed that haplotypes AA and GG were statistically significant in disease association with more haplotype AA in AD (frequency 0.4113 and $P = 8.09\times10^{-7}$ with $\chi^2 = 24.34$) and more haplotype GG in normal controls (frequency 0.6592 and $P = 1.72\times10^{-7}$ with $\chi^2 = 27.33$).

We investigated whether these candidate genes could be replicated when MCI individuals were compared to normal controls. Apart from rs2387976 in *APOE* ε4 non-carrier subgroup, all other SNPs could reach the significant level of $P < 0.05$ (Table **2**). Furthermore, the frequency of these SNPs in MCI patients were between that of normal controls and AD patients (Table **2**), showing a trend of increased frequency from controls to MCI and finally to AD. All these evidences suggested that these identified SNPs are strong candidates for quantitative trait analyses.

rs6816078 and rs28604990-rs7955747 are Associated with CSF Biomarkers in *APOE* ε4 Non-Carriers Subgroup

Next, quantitative-trait analyses were performed to further investigate whether these candidate SNPs were associated with AD. rs2075650 was excluded in this part because it has been demonstrated to be associated with $A\beta_{1\text{-}42}$ in a ADNI study [38] and its association with AD has been widely reported.

Because CSF biomarker and brain morphometric volume data were not detected in 12 individuals with genotypic data of rs2387976, its linked SNP rs6076364 was chosen to represent the haplotype constituted by them because of the extremely high r^2 value of 1. The haplotype in *ZNF827* was represented by the most significant SNP rs6816078 in *APOE* ε4 non-carriers.

First the relationship of these SNPs or haplotypes and CSF biomarker indices were analyzed at screening time point (Supplementary Table **1-3**). In all participants without considering *APOE* ε4 status, $A\beta_{42}$, t-tau and p-tau_{181p} CSF levels and t-tau/$A\beta_{42}$ ratio of carriers of rs12822144 genotypes GG and AG were significantly different from that of carriers of genotype AA (dominant model) (Supplementary Table **1**). In *APOE* ε4 non-carriers of all participants, there were significant differences in CSF $A\beta_{42}$ level, t-tau/$A\beta_{42}$ and p-tau_{181p}/$A\beta_{42}$ ratios between carriers of genotype CC and genotypes AA and AC of rs6816078 (recessive model), and in CSF t-tau level, t-tau/$A\beta_{42}$ and p-tau_{181p}/$A\beta_{42}$ ratios between carriers of genotypes GG and AG and genotype AA homozygote of rs28604990 (dominant model) (Supplementary Table **1**). When we carried out these comparisons only in AD patients, CSF p-tau_{181p} levels and p-tau_{181p}/$A\beta_{42}$ ratios of carriers of genotype CC of rs6816078were significantly higher than that of genotypes AA and AC carriers (recessive model) (Supplementary Table **2**). When normal controls were analyzed, p-tau_{181p}/$A\beta_{42}$ ratios of carriers of genotype CC of rs6076364 were significantly different from that of genotypes CC and TC carriers (recessive model) (Supplementary Table **3**).

Further, in normal controls non-carrying *APOE* ε4, there were significant differences of CSF $A\beta_{42}$ levels at 12-month and 36-month time points between rs6816078 genotypes AA

and AC carriers and CC carriers (recessive model) (Fig. **3A**). The differences were significant even after multiple dataset correction.

We further analyzed whether these SNPs or haplotypes influenced the CSF biomarker indices time-dependently. The analysis demonstrated that the CSF $A\beta_{42}$ level of rs6816078 genotype CC carriers in normal controls non-carrying *APOE* ε4 decreased over time from screening to 36-month time point, whereas p-tau_{181p}/$A\beta_{42}$ ratio increased (Fig. **3A** and **3B**). The *P* value was 0.131 for genotype CC carriers, post hoc analysis revealed that the difference of CSF $A\beta_{42}$ levels between screening and 36-month time point almost reached the significant threshold ($P = 0.052$). However, the *P* value was 0.423 for genotypes AA and AC carriers. For p-tau_{181p}/$A\beta_{42}$ ratio, the *P* value was 0.07 for genotype CC carriers, and post hoc analysis revealed that the differences of p-tau_{181p}/$A\beta_{42}$ between screening and 12-month time points and between screening and 36-month time points almost reached the significant threshold ($P = 0.067$ and 0.058 respectively). However, the *P* value was 0.703 for genotype AA and AC carriers.

Moreover, the CSF p-tau_{181p} level and p-tau_{181p}/$A\beta_{42}$ ratio of rs28604990-rs7955747 risk haplotypes AA and AG carriers in normal controls non-carrying *APOE* ε4 increased over time (Fig. **3C** and **3D**). The *P* values were 0.004 for p-tau_{181p} and 0.043 for p-tau_{181p}/$A\beta_{42}$ ratio in carriers of risk haplotypes. Post hoc analyses revealed that the differences of p-tau_{181p} between screening and 12-month time points and between screening and 36-month time points and the difference of p-tau_{181p}/$A\beta_{42}$ ratio between screening and 36-month time points were statistically significant ($P < 0.05$). The differences were still significant even after multiple dataset correction. However, the *P* values were 0.907 and 0.798 for p-tau_{181p} and p-tau_{181p}/$A\beta_{42}$ ratio respectively in AA and AG non-carriers.

rs6076364 is Associated with Brain Atrophy

AD patients of *APOE* ε4 non-carriers carrying genotype TT of rs6076364 were found to be prone to brain atrophy, especially the estimated intracranial volume (Table **3**). The differences of estimated intracranial volumes between genotype TT carriers and CC and TC carriers were statistically

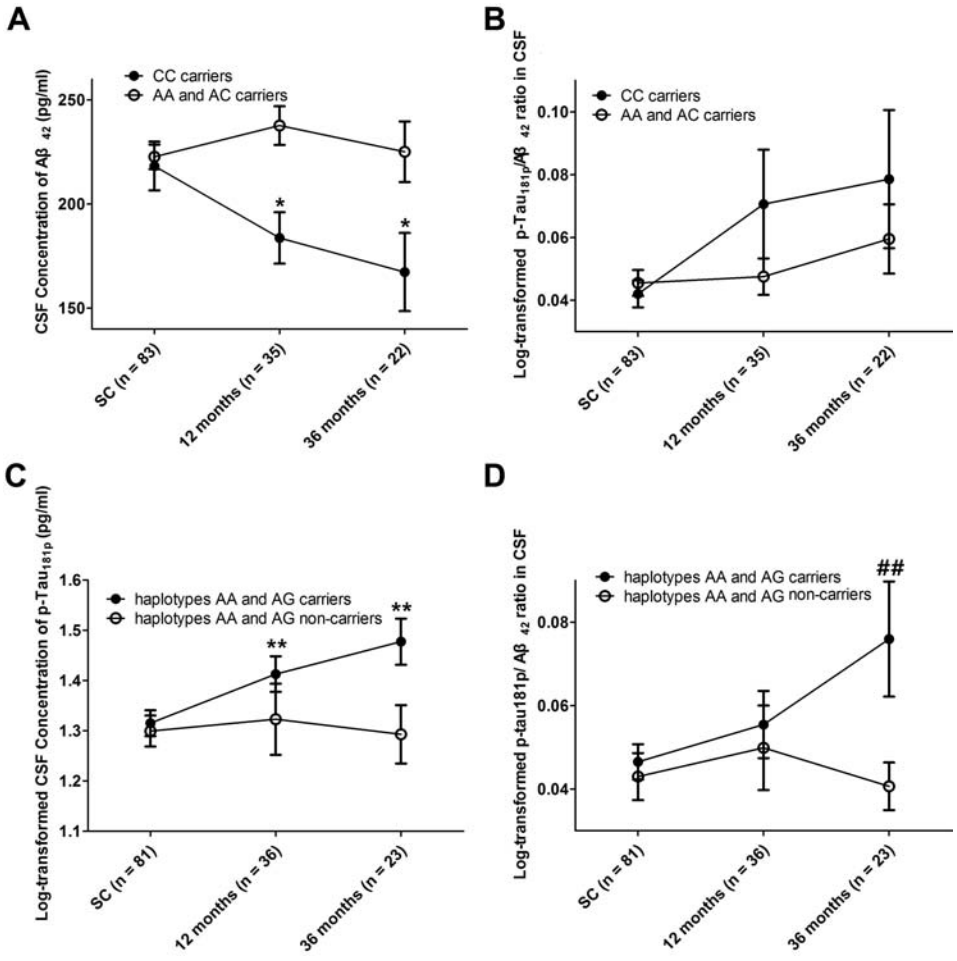

Fig. (3). Comparisons of CSF indices in different genotypes of rs6816078 and rs28604990-rs7955747 in normal controls. A and B: comparisons of CSF $A\beta_{42}$ level (**A**) and log-transformed p-tau_{181p}/$A\beta_{42}$ ratio (**B**) of different time points between rs6816078 genotype CC carriers and genotypes AA and AC carriers. C and D: comparisons between CSF levels of p-tau_{181p} (**C**) and log-transformed p-tau_{181p}/$A\beta_{42}$ ratio (**D**) of different time points in rs28604990-rs7955747 risk haplotypes AA and AG carriers and non-carriers. Black and white circles with bars represent mean ± SEM. *: $P < 0.05$, **: $P < 0.01$ *versus* corresponding points in AA and AC carriers. ##: $P < 0.01$ *versus* SC point. SC represents screening level. The differences were significant after multiple dataset correction.

Table 3. Comparisons between rs6076364 genotypes of AD patients in *APOE* ε4 non-carriers.

Time Point	Genotypes	Whole Brain Volume, mean ± SEM (n)	*P* Value	Estimated Intracranial Volume, mean ± SEM (n)	*P* Value
screening	TT carriers	954126.93 ± 17697.11 (11)	0.064	1395333.33 ± 18512.97 (11)	0.012
	CC and CT carriers	1005546.65 ± 12913.15 (13)		1464166.67 ± 18541.89 (13)	
6 months	TT carriers	957204.92 ± 20327.48 (9)	0.099	1399166.67 ± 22778.86 (9)	0.028
	CC and CT carriers	1006645.76 ± 13116.29 (9)		1467962.96 ± 19136.46 (9)	
12 months	TT carriers	972078 ± 25191.07 (8)	0.444	1417500 ± 31552.11 (8)	0.388
	CC and CT carriers	998381.06 ± 13244.35 (8)		1461458.33 ± 19828.59 (8)	
24 months	TT carriers	949396.7 ± 23598.9 (4)	0.187	1404000 ± 26465.91 (4)	0.231
	CC and CT carriers	992548.82 ± 15094.18 (6)		1457948.72 ± 21373.8 (6)	

significant at screening and 6-month time points, and the statistically significant difference of estimated intracranial volumes between genotype TT carriers and CC and TC carriers at screening time point was excluded after multiple dataset correction. The differences of whole brain volumes between genotype TT carriers and CC and TC carriers at screening and 6-month time points almost reached the statistical threshold.

Predicting Power of AD Diagnostic or Brain Morphometric Volume-Associated Haplotype Blocks in *APOE* ε4 Non-Carriers

The above results indicated that the identified three haplotypes were strongly associated with AD in *APOE* ε4 non-carriers, we next investigated whether these haplotype blocks could be combined together to predict the incidence of AD in *APOE* ε4 non-carriers. After adjusted for sex, age and education, all haplotype blocks were entered into the eventual equation and reached statistically significant threshold. The pseudo-R^2 was 0.393, indicating a moderate goodness of fit. The positive β coefficients of the three variables in the equation suggested the additive AD-causing effects of these haplotype blocks (Table **4**).

SNPs Identified in *APOE* ε4 Non-Carriers Interacts with *APOE* ε4

To further analyze the relationship of these SNPs with *APOE* ε4, the interactions between identified SNPs and *APOE* ε4 were assessed. After adjusting for SNP genotypes, *APOE* ε4 status, sex, age and education years, risk alleles of all SNPs identified in *APOE* ε4 non-carriers could take their effects by interacting with *APOE* ε4 status (Table **5**).

DISCUSSION

There is plenty of evidence demonstrating the effects of *APOE* ε4 in the pathological process of AD [4; 39-41]. The distinct phenotypes of AD without *APOE* ε4 have also been characterized [5-8, 42], however, the factors behind it is still uncovered and almost all of them are uncorrelated with quantitative traits, such as CSF biomarkers Aβ and p-tau. Moreover, even though several GWASs exclusively for quantitative traits of AD have been carried out and have identified many correlated SNPs [38, 43], many of them are not necessarily associated with AD. The present study aimed to differentiate influential genetic risk factors and to screen candidate SNPs associated with AD in *APOE* ε4 carriers and non-carriers respectively by combining genome association analysis and the analyses of quantitative traits such as CSF biomarkers and general brain morphometric volumes.

Without considering *APOE* ε4 status, two SNPs were screened. One of them, rs2075650, has been widely reported to be related to the quantitative traits of AD in ADNI cohort [38, 43]. Our analysis also revealed that carriers of risk allele of this SNP showed earlier onsets of AD symptoms (data not shown), which is consistent with other studies [44, 45]. Thus our data further confirm that this SNP is a risk factor of AD.

Table 4. Logistic regression model for predicting power of haplotype blocks in *APOE* ε4 non-carriers.

Variables	β Coefficient	Wald	OR (95% CI)[a,b]	*P* Value[b]
rs6816078-rs11930385 or rs6816078	1.18	17.69	3.27(1.88-5.68)	2.6×10^{-5}
rs28604990-rs7955747	0.79	5.85	2.20(1.16-4.16)	0.016
rs6076364-rs2387976 or rs6076364	1.16	14.85	3.18(1.76-5.72)	1.16×10^{-4}

[a]Abbreviation: OR, odds ratio.
[b]Adjusted for age of onset, sex, and education.

Table 5. Multi-covariate logistic regression analyses for SNPs identified in GWA analyses.

SNP	OR (95% CI) for SNP×*APOE* ε4 status[a,b]	*P* value for SNP×*APOE* ε4 status[b]
rs2075650	3.658 (0.960-13.950)	0.058
rs12822144	1.109 (0.573-2.146)	0.758
rs6816078	2.885 (1.489-5.589)	1.69×10^{-3}
rs28604990	0.239 (0.125-0.460)	1.75×10^{-5}
rs11930385	2.630 (1.323-5.230)	5.82×10^{-3}
rs7955747	0.235 (0.118-0.469)	3.94×10^{-5}
rs2387976	3.304 (1.684-6.485)	5.13×10^{-4}
rs6076364	3.333 (1.708-6.505)	4.18×10^{-4}

[a]Abbreviation: OR: odds ratio; CI: confidence interval.
[b]Adjusted for SNP genotypes, *APOE* ε4 status, sex, age and education years.

When the participants were divided into *APOE* ε4 carriers and non-carriers, the susceptibility SNPs identified without considering *APOE* ε4 status did not replicate in any subgroups. In *APOE* ε4 non-carriers, five SNPs were initially screened to associate with AD and four of them could be replicated in MCI patients. The risk allele frequencies of these SNPs showed an increasing trend from controls to MCI and to AD, implicating the significance of these SNPs. Further these SNPs and haplotypes constituted by these SNPs were shown to be associated with CSF $A\beta_{42}$ level, p-tau_{181p} level, p-tau_{181p}/$A\beta_{42}$ ratio, or brain atrophy in *APOE* ε4 non-carriers. Moreover, these SNPs were shown to take additive effects on AD incidence. These results indicate that these SNPs and haplotypes are strongly associated with AD in *APOE* ε4 non-carriers.

ZNF827, which rs6816078-rs11930385 is located in, is a member of ZNFs. ZNFs are typical transcriptional regulators [46]. Several ZNFs have been identified to correlate with AD [45, 47]. One brain expression GWAS demonstrates that minor allele of rs2387976 is associated with reduced transcript of *NANP* [48], implicating the altered expression of *NANP* in AD patients. Our results showed that these SNPs and haplotypes were associated with CSF $A\beta_{42}$, p-tau_{181p} levels or brain atrophy, indicating that the modification of these pathological processes through transcriptional and/or gene expression regulation could be involved in their roles in AD.

The identified SNPs in *APOE* ε4 non-carriers could not be replicated in *APOE* ε4 carriers, and could not even reach a nominal significant level. Many SNPs which were nominally significant in *APOE* ε4 non-carriers could not be replicated in *APOE* ε4 carriers either (data not shown). Moreover, the associations of these SNPs with quantitative traits were absent in *APOE* ε4 carriers (data not shown). This is the same to *APOE* ε4 carrier subgroup whose association results could not be replicated in *APOE* ε4 non-carriers (data not shown). One GWAS of Caribbean Hispanic individuals has revealed similar phenomenon [19]. These evidences implicate that the associations between SNPs identified via previous GWAS and AD-associated quantitative traits may be masked by *APOE* ε4 status. Moreover, logistic regression analysis showed that these SNPs did interact with *APOE* ε4 status, but not the SNPs identified without stratification on *APOE* genotype. All these data support the notion that these SNPs play their roles based on the carrier status of *APOE* ε4. Our analysis are consistent with other reports showing distinct mechanisms involved in AD dependent on *APOE* ε4 status [13, 14], further supporting the view that different genetic forms or subtypes of Alzheimer's disease may have specific molecular mechanisms [49].

In conclusion, by combining genome association analysis and quantitative trait analyses, the present study identified the unique susceptibility SNPs and haplotypes for AD negative of *APOE* ε4, suggesting that different signaling pathways and biological processes may be involved in the pathology of AD in *APOE* ε4 carriers and non-carriers. The susceptible SNPs and haplotypes identified in current study may promote the unraveling of genetic risks and distinct pathology of AD patients without *APOE* ε4 and may improve the understanding of the pathogenesis of AD. Because most of identified SNPs in current study are not covered in some other databases and no more databases have such comprehensive data as ADNI, the replication is not able to be carried out currently. However, the quantitative trait analyses provided the further evidence and thus partially offset the restricted statistical power of genome-wide screening. Moreover, our study provides a new way to identify susceptible loci and complements previous GWASs by combining genome association analysis and quantitative trait analyses.

CONFLICT OF INTEREST

The authors have not submitted the manuscript elsewhere and declare that they have no competing interests. There isn't any interest to disclose.

ACKNOWLEDGEMENTS

Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: Alzheimer's Association; Alzheimer's Drug Discovery Foundation; BioClinica, Inc.; Biogen Idec Inc.; Bristol-Myers Squibb Company; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; GE Healthcare; Innogenetics, N.V.; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Medpace, Inc.; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Synarc Inc.; and Takeda Pharmaceutical Company. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern

California Institute for Research and Education, and the study is coordinated by the Alzheimer's Disease Cooperative Study at the University of California, San Diego. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of California, Los Angeles. This research was also supported by NIH grants P30 AG010129 and K01 AG030514. Acknowledgement list for ADNI publications according to the ADNI publication policy could be accessed from the following link: http://adni.loni.ucla.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

The analysis of the data and the writing of the report were supported by International Science & Technology Cooperation Program of China (2011DFA33180) and National Comprehensive Technology Platforms for Innovative Drug R&D of China (No.2012ZX09303-003).

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers web site along with the published article.

REFERENCES

[1] Strittmatter WJ, Roses AD. Apolipoprotein E and Alzheimer disease. Proc Natl Acad Sci USA 92(11): 4725-7 (1995).

[2] Coon KD, Myers AJ, Craig DW, Webster JA, Pearson JV, Lince DH, *et al.* A high-density whole-genome association study reveals that APOE is the major susceptibility gene for sporadic late-onset Alzheimer's disease. J Clin Psychiatry 68(4): 613 (2007).

[3] Davidson Y, Gibbons L, Pritchard A, Hardicre J, Wren J, Stopford C, *et al.* Apolipoprotein E epsilon4 allele frequency and age at onset of Alzheimer's disease. Dement Geriatr Cogn Disord 23(1): 60-6 (2007).

[4] Kim J, Basak JM, Holtzman DM. The role of apolipoprotein E in Alzheimer's disease. Neuron 63(3): 287-303 (2009).

[5] Marra C, Bizzarro A, Daniele A, De Luca L, Ferraccioli M, Valenza A, *et al.* Apolipoprotein E epsilon4 allele differently affects the patterns of neuropsychological presentation in early- and late-onset Alzheimer's disease patients. Dement Geriatr Cogn Disord 18(2): 125-31 (2004).

[6] van der Vlies AE, Pijnenburg YA, Koene T, Klein M, Kok A, Scheltens P, *et al.* Cognitive impairment in Alzheimer's disease is modified by APOE genotype. Dement Geriatr Cogn Disord 24(2): 98-103 (2007).

[7] Wolk DA, Dickerson BC, Weiner M, Aiello M, Aisen P, Albert MS, *et al.* Apolipoprotein E (APOE) genotype has dissociable effects on memory and attentional–executive network function in Alzheimer's disease. Proc Natl Acad Sci USA 107(22): 10256-61 (2010).

[8] Scarmeas N, Anderson K, Hilton J, Park A, Habeck C, Flynn J, *et al.* APOE-dependent PET patterns of brain activation in Alzheimer disease. Neurology 63(5): 913-15 (2004).

[9] Murphy EA, Roddey JC, McEvoy LK, Holland D, Hagler DJ, Jr., Dale AM, *et al.* CETP polymorphisms associate with brain structure, atrophy rate, and Alzheimer's disease risk in an APOE-dependent manner. Brain Imaging Behav 6(1): 16-26 (2012).

[10] Rodríguez E, Mateo I, Infante J, Llorca J, Berciano J, Combarros O. Cholesteryl ester transfer protein (CETP) polymorphism modifies the Alzheimer's disease risk associated with APOE ε4 allele. J Neurol 253(2): 181-85 (2006).

[11] Feuk L, Prince JA, Breen G, Emahazion T, Carothers A, St Clair D, *et al.* apolipoprotein-E dependent role for the FAS receptor in early onset Alzheimer's disease: finding of a positive association for a polymorphism in the TNFRSF6 gene. Hum Genet 107(4): 391-6 (2000).

[12] Beyer K, Lao JI, Gomez M, Riutort N, Latorre P, Mate JL, *et al.* Alzheimer's disease and the cystatin C gene polymorphism: an association study. Neurosci Lett 315(1-2): 17-20 (2001).

[13] Wijsman EM, Pankratz ND, Choi Y, Rothstein JH, Faber KM, Cheng R, *et al.* Genome-wide association of familial late-onset Alzheimer's disease replicates BIN1 and CLU and nominates CUGBP2 in interaction with APOE. PLoS Genet 7(2): e1001308 (2011).

[14] Rhinn H, Fujita R, Qiang L, Cheng R, Lee JH, Abeliovich A. Integrative genomics identifies APOE epsilon4 effectors in Alzheimer's disease. Nature 500(7460): 45-50 (2013).

[15] Bertram L, McQueen MB, Mullin K, Blacker D, Tanzi RE. Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database. Nat Genet 39(1): 17-23 (2007).

[16] Maloney B, Ge YW, Petersen RC, Hardy J, Rogers JT, Perez-Tur J, *et al.* Functional characterization of three single-nucleotide polymorphisms present in the human APOE promoter sequence: Differential effects in neuronal cells and on DNA-protein interactions. American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics 153B(1): 185-201 (2010).

[17] Lahiri DK, Maloney B, Zawia NH. The LEARn model: an epigenetic explanation for idiopathic neurobiological diseases. Molecular psychiatry 14(11): 992-1003 (2009).

[18] Reiman EM, Webster JA, Myers AJ, Hardy J, Dunckley T, Zismann VL, *et al.* GAB2 alleles modify Alzheimer's risk in APOE epsilon4 carriers. Neuron 54(5): 713-20 (2007).

[19] Lee JH, Cheng R, Barral S, Reitz C, Medrano M, Lantigua R, *et al.* Identification of novel loci for Alzheimer disease and replication of CLU, PICALM, and BIN1 in Caribbean Hispanic individuals. Arch Neurol 68(3): 320-8 (2011).

[20] Blennow K, Hampel H, Weiner M, Zetterberg H. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. Nat Rev Neurol 6(3): 131-44 (2010).

[21] Kolsch H, Jessen F, Wiltfang J, Lewczuk P, Dichgans M, Kornhuber J, *et al.* Influence of SORL1 gene variants: association with CSF amyloid-beta products in probable Alzheimer's disease. Neurosci Lett 440(1): 68-71 (2008).

[22] Kauwe JS, Wang J, Mayo K, Morris JC, Fagan AM, Holtzman DM, *et al*. Alzheimer's disease risk variants show association with cerebrospinal fluid amyloid beta. Neurogenetics 10(1): 13-17 (2009).

[23] Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, *et al*. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet 41(10) : 1088-93 (2009).

[24] Hollingworth P, Harold D, Sims R, Gerrish A, Lambert JC, Carrasquillo MM, *et al.* Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet 43(5): 429-35 (2011).

[25] Petersen RC, Aisen PS, Beckett LA, Donohue MC, Gamst AC, Harvey DJ, *et al.* Alzheimer's Disease Neuroimaging Initiative (ADNI): clinical characterization. Neurology 74(3): 201-9(2010).

[26] World Medical A. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 310(20): 2191-4 (2013).

[27] Leow AD, Klunder AD, Jack Jr CR, Toga AW, Dale AM, Bernstein MA, *et al.* Longitudinal stability of MRI for mapping brain change using tensor-based morphometry. Neuroimage 31(2): 627-40 (2006).

[28] Jack CR, Jr., Bernstein MA, Fox NC, Thompson P, Alexander G, Harvey D, *et al.* The Alzheimer's Disease Neuroimaging Initiative (ADNI): MRI methods. J Magn Reson Imaging 27(4): 685-91 (2008).

[29] Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21(2): 263-5 (2005).

[30] Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81(3): 559-75 (2007).

[31] Lambert JC, Grenier-Boley B, Chouraki V, Heath S, Zelenika D, Fievet N, *et al*. Implication of the immune system in Alzheimer's disease: evidence from genome-wide pathway analysis. J Alzheimers Dis 20(4): 1107-18 (2010).

[32] Zhou Z, Yuan Q, Mash DC, Goldman D. Substance-specific and shared transcription and epigenetic changes in the human hippocampus chronically exposed to cocaine and alcohol. Proc Natl Acad Sci USA 108(16): 6626-31 (2011).

[33] Krejci J, Uhlirova R, Galiova G, Kozubek S, Smigova J, Bartova E. Genome-wide reduction in H3K9 acetylation during human em-

bryonic stem cell differentiation. J Cell Physiol 219(3): 677-87 (2009).

[34] Han S, Lee KM, Park SK, Lee JE, Ahn HS, Shin HY, *et al.* Genome-wide association study of childhood acute lymphoblastic leukemia in Korea. Leuk Res 34(10): 1271-4 (2010).

[35] Li W, Antuono PG, Xie C, Chen G, Jones JL, Ward BD, *et al.* Aberrant functional connectivity in Papez circuit correlates with memory performance in cognitively intact middle-aged APOE4 carriers. Cortex 57C: 167-76 (2014).

[36] Martinez-Garcia A, Aldudo J, Recuero M, Sastre I, Vilella-Cuadrada E, Rosich-Estrago M, *et al.* Presenilin 1 polymorphism associated with Alzheimer's disease in apolipoprotein E4 carriers. Dement Geriatr Cogn Disord 26(5): 440-4 (2008).

[37] Matsubara-Tsutsui M, Yamagata H, Morishima A, Nakura J, Mitsuda N, Kamino K, *et al.* The 4,752 C/T polymorphism in the presenilin 1 gene increases the risk of Alzheimer's disease in apolipoprotein E4 carriers. Intern Med 41(10): 823-8 (2002).

[38] Kim S, Swaminathan S, Shen L, Risacher SL, Nho K, Foroud T, *et al.* Genome-wide association study of CSF biomarkers Abeta1-42, t-tau, and p-tau181p in the ADNI cohort. Neurology 76(1): 69-79 (2011).

[39] Olichney J, Hansen L, Galasko D, Saitoh T, Hofstetter C, Katzman R, *et al.* The apolipoprotein E epsilon 4 allele is associated with increased neuritic plaques and cerebral amyloid angiopathy in Alzheimer's disease and Lewy body variant. Neurology 47(1): 190-96 (1996).

[40] Bondi MW, Salmon DP, Monsch AU, Galasko D, Butters N, Klauber MR, *et al.* Episodic memory changes are associated with the APOE-epsilon 4 allele in nondemented older adults. Neurology 45(12): 2203-6 (1995).

[41] Sunderland T, Mirza N, Putnam KT, Linker G, Bhupali D, Durham R, *et al.* Cerebrospinal fluid β-amyloid$_{1–42}$ and tau in control subjects at risk for Alzheimer's disease: The effect of APOE ε4 allele. Biol Psychiatry 56(9): 670-76 (2004).

[42] van der Flier WM, Pijnenburg YA, Fox NC, Scheltens P. Early-onset versus late-onset Alzheimer's disease: the case of the missing APOE varepsilon4 allele. Lancet Neurol 10(3): 280-8 (2011).

[43] Shen L, Kim S, Risacher SL, Nho K, Swaminathan S, West JD, *et al.* Whole genome association study of brain-wide imaging phenotypes for identifying quantitative trait loci in MCI and AD: A study of the ADNI cohort. Neuroimage 53(3): 1051-63 (2010).

[44] Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Jr., Rimmler JB, *et al.* Apolipoprotein E, survival in Alzheimer's disease patients, and the competing risks of death and Alzheimer's disease. Neurology 45(7): 1323-8 (1995).

[45] Kamboh MI, Barmada MM, Demirci FY, Minster RL, Carrasquillo MM, Pankratz VS, *et al.* Genome-wide association analysis of age-at-onset in Alzheimer's disease. Molecular psychiatry 17(12): 1340-6 (2012).

[46] Sharma S, Dimasi D, Higginson K, Della NG. RZF, a zinc-finger protein in the photoreceptors of human retina. Gene 342(2): 219-29 (2004).

[47] Hollingworth P, Sweet R, Sims R, Harold D, Russo G, Abraham R, *et al.* Genome-wide association study of Alzheimer's disease with psychotic symptoms. Molecular psychiatry 17(12): 1316-27 (2012).

[48] Zou F, Chai HS, Younkin CS, Allen M, Crook J, Pankratz VS, *et al.* Brain expression genome-wide association study (eGWAS) identifies human disease-associated variants. PLoS Genet 8(6): e1002707 (2012).

[49] Swarup V, Geschwind DH. Alzheimer's disease: From big data to mechanism. Nature 500(7460): 34-5 (2013).

Received: December 16, 2014 **Revised: January 28, 2015** **Accepted: February 02, 2015**